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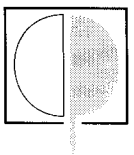

Tolerance of lemons and the Mediterranean fruit fly to carbonyl sulfide quarantine fumigation

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Lemons (*Citrus limon* Burm.) were evaluated for their tolerance to carbonyl sulfide (COS) fumigation and the influence of COS on market quality. At 70 mg l^{-1} no significant deleterious changes occurred in market quality up to a fumigation duration of 8 h and only a slight amount of peel injury was observed after 12 h. Longer fumigations lead to the presence of an offensive off-odor in the juice as well as to increasing rind injury. A test of the sensitivity of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) to COS indicated that long fumigation times (more than 8 h) will likely be required to achieve a degree of mortality sufficient for quarantine purposes for this insect. The tolerance of lemons to COS observed in this study suggests that COS is suitable for use as a quarantine treatment for this commodity. Its adoption, however, for this purpose will be hampered by the relatively long fumigation times needed and the odor that temporarily accompanies the fruit following fumigation. Published by Elsevier Science Ltd

Keywords: phytotoxicity; *Ceratitis capitata*; *Citrus limon*

The treatment of fresh commodities with chemical fumigants is commonly practiced as a means of insect disinfestation. Methyl bromide, the most commonly used fumigant for this purpose, has been classified as an ozone depleter and, as such, may be withdrawn from use in the near future. Although non-chemical alternatives, such as heat (Lay-Yee and Rose, 1994; McGuire, 1991; Sharp and McGuire, 1996; Shellie and Mangan, 1995) and cold (Hill *et al.*, 1988; Houck *et al.*, 1990), have shown promise in the disinfestation of some fresh commodities, these treatments require longer treatment times than does methyl bromide and would require the construction of expensive new facilities, resulting in higher treatment costs. Capital and operating costs of forced air/vapor heat treatment, for instance, have been estimated to be between six and seven times greater than conventional methyl bromide fumigation (EPA, 1996). Combination treatments, such as hot air/controlled atmosphere treatments (Neven and Mitcham, 1996), may offer a means to reduce treatment times for some commodities, although these treatments, due to their added complexity, are likely to be costly to implement. An alternative chemical fumigant may offer a less expensive and more practical alternative to methyl bromide.

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Carbonyl sulfide (COS) is a gas that in pure form is colorless and odorless. The compound is well known, being used in the production of thiocarbamate herbicides and produced as a by-product of carbon disulfide production (EPA, 1994). Also, carbonyl sulfide is naturally present in the environment and is an abundant form of sulfur in the atmosphere. Plants are able to metabolize COS, presumably due to the enzymatic activity of carbonic anhydrase (Protoschill-Krebs and Kesselmeier, 1992) and they are also able to synthesize it (Feng and Hartel, 1996). The existence of a large natural flux of COS in the environment will probably minimize the impact of manmade releases of this compound. Recently, COS was patented as an agent that is able to control insects and mites in postharvest commodities (Banks *et al.*, 1993). The authors of the patent found COS to be toxic to a wide range of insects at concentrations comparable to a number of other known fumigants. The effectiveness of COS in the control of insects, combined with its environmentally friendly characteristics, make it a fumigant that holds promise as an alternative to methyl bromide.

Although COS has been patented (Banks *et al.*, 1993), and has been tested and found to be useful as a fumigant for dry commodities (Plarre and Reichmuth, 1996; Zettler *et al.*, 1997), its effectiveness in the disinfestation of fresh commodities has not been well described. In this paper, research concerning the

tolerance of California lemons to COS is presented along with data concerning the susceptibility of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann), a postharvest pest of great concern in California, to COS fumigation.

Materials and methods

Evaluation of lemon quality following fumigation

All work on fruit quality was conducted at the USDA/ARS laboratory in Fresno, CA. Commercially packed yellow lemons (cv. Eureka) were obtained locally from a packing plant in Ivanhoe, CA and stored at 7°C and 65% RH to await fumigation. Fruit size was 165 fruit per box (100 g per fruit). Prior to fumigation the lemons were removed from cold storage, randomized, and allowed to warm to ambient temperature for 12 h at 23°C, the fumigation temperature. All fumigations were completed within 1 week.

Fumigation treatments were performed in 29.5 l glass jars enclosed at the top with a metal plate. Attached to the plate was a small electric fan, which functioned to assure mixing of the air and fumigant within the jar. The fan was operated during the first hour of fumigation and immediately prior to gas sampling. A single port in the metal plate allowed for both the introduction of COS into the fumigation jar and withdrawal of a gas sample for the determination of the COS concentration.

Fumigant concentration at the start of the treatment was 70 mg l^{-1} and treatment durations were 4, 6, 8, 12, 16 or 20 h. These time/concentration combinations were chosen in an attempt to include dosages that were previously found to be effective against pupae, eggs and larvae of Queensland fruit fly (*Bactrocera tyroni* Froggat), the only previous test of COS against fruit fly that we are aware of (Banks *et al.*, 1993). Control fruit were similarly held in a sealed jar as treated fruit. All treatments and controls were replicated in three separate tests using the same lot of fruit.

To begin the fumigation treatment, 65 lemons were placed in each fumigation jar to give a 25% load by volume. After sealing the jars, COS (Aldrich Chemical, St Louis, MO) was introduced into the jars by a gas-tight syringe. Concentrations of COS were then immediately determined by withdrawing a gas sample using a gas-tight syringe and injecting it into a gas chromatograph (GC8A, Shimadzu Scientific) equipped with a 1 ml gas sample loop, TCD detector and a 76.2 cm × 0.32 cm Chromatosil 330 column (Supelco Inc., Bellefonte, PA). Oven and detector temperatures were 50 and 70°C, respectively, and helium flow was maintained through the column at 60 ml min^{-1}. COS concentration was calculated from a standard curve. At the end of the treatment the concentration of COS was remeasured to determine the concentration × time (CXT) dose. Since the decline in COS concentration during fumigation was not linear (*Figure 1*), adjustments were made in the CXT values by utilizing measurements taken every 4 h. Following fumigation, the jar was unsealed and

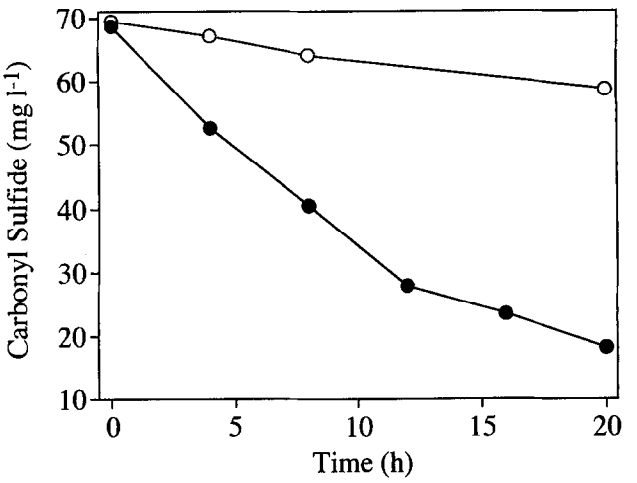

Figure 1. Carbonyl sulfide concentration in a fumigation chamber as related to duration of fumigation. Open circles are measurements from empty fumigation chambers and closed circles from chambers containing a 25% load by volume of lemons. Each data point was derived from three replicates

forcibly aerated for 15 min, with the jars standing in a hood with an airflow of 0.64 m s^{-1}. After 2 h the fruits were removed from the jars, placed into plastic mesh baskets and allowed to aerate for a further 24 h in the hood. To assess initial coloration changes due to fumigation, surface color was evaluated at three equatorial sites on every fruit using a Minolta CR-200 chroma meter (Minolta, Ramsey, NJ). Fruits were then individually weighed, packed into boxes and stored at 7°C (65% RH).

Following 4 weeks of storage at 7°C and 65% RH plus 12 additional hours at 22°C, the lemons were all individually weighed and evaluated for surface injury. Injury was quantified by visibly assessing the number and size of lesions and other miscellaneous injuries and placing each fruit into one of six classes. The injury classes and associated multiplication factors were: healthy (0), very slight (1), slight (2), moderate (3), severe (4) and very severe (5). A numerically weighted injury index value was then calculated for each treatment by multiplying the number of fruit in each class by the appropriate multiplication factor and dividing by the number of fruit in each class. Pitting was the major injury observed and the amount of surface area covered by pitting per injury class was: healthy (0), very slight (5% or less), slight (5–15%), moderate (15–25%), severe (25–50%) and very severe (> 50%). Fruit given ratings of moderate, severe and very severe were considered to be unmarketable. Firmness of 25 fruit per replication was measured at one equatorial site with an Instron Model 4201 (Instron, Canton, MA) fitted with a compression anvil 3 cm in diameter. The amount of movement by the anvil, traveling at 25 mm min^{-1}, prior to achieving a compression force of 1 kg, was the measure of firmness. Twenty fruit per replication were measured for firmness. Color was again evaluated on every fruit in the same manner as the prior color measurement in order to determine if color had changed during storage. Ten fruit from each replication were juiced and the juice used to measure titratable acidity by means of NaOH titration. Soluble solids were quantified from the same juice using a

handheld refractometer and pH was determined by a pH meter.

Mediterranean fruit fly response to COS

The feasibility of a new fumigant must take into account the dose needed and exposure times necessary to kill the target pest. The postharvest pest of greatest threat in California is the Mediterranean fruit fly (MFF). As no data were available on the tolerance of this insect to COS, tests were conducted to determine the susceptibility of MFF to COS. Laboratory-reared MFF pupae were provided by the USDA/ARS laboratory in Honolulu, Hawaii. Desired life stages (eggs, first and third instars) were obtained from these pupae as previously described (Jang, 1996). Fumigation treatment of the insects was conducted at the USDA/ARS laboratory in Hilo, Hawaii.

Fumigation was conducted in wide-mouth 975 ml mason jars sealed with canning jar lids fitted with a single port in the lid for the introduction or sampling of the gas. The concentration of COS in the jars was determined by withdrawing a headspace sample using a gas-tight syringe and injecting it into a gas chromatograph. Chromatography equipment and run conditions were as described previously for the fruit quality experiments. Carbonyl sulfide concentrations were measured immediately following both the initiation and completion of fumigation in order to calculate the concentration × time (CXT) dose.

Eggs (50% developed) and first instar larvae were fumigated in mason jars on moistened blotter paper. During fumigation the third instars were placed in small screen enclosures, containing a piece of moist blotter paper. In the second experiment, first and third instars were placed onto moistened blotter paper and then into plastic vials with screened ends for fumigation. In all cases, 100 eggs or 50 third instar larvae were used per treatment. For first instar larvae, the larvae were collected and applied to the blotter paper with a paint brush and the initial counts were not known. At the end of the treatment, the jars were unsealed and the COS flushed out using 5 min of positive airflow. After a further 10 min of aeration in the hood, the insects were removed from the jars. Treated eggs and moistened blotter paper were then placed into petri plates, sealed into plastic bags and allowed to incubate for 3–5 days at 26°C to allow for hatch to occur. Survivorship was indicated by those eggs that hatched into larvae. Natural mortality was adjusted for in the treated eggs (Abbott, 1925). Larvae, once removed from the fumigation jars, were placed on an artificial diet (Tanaka *et al.*, 1969) and incubated for 7–10 days at 26°C to allow for development. Pupation was then determined, with percent mortality based on an estimated population derived from the number of pupae in the controls for the first instars and based on actual counts for the third instars. As with the eggs, natural mortality was adjusted for using Abbot's correction for the third instar larvae. Controls were placed in jars containing no COS but sealed for an equal duration to that of the maximum treatment time.

To estimate the potential influence of the lemon fruit on the effectiveness of COS fumigation, a small hole was made with a cork borer into the stylar end of the fruit, through which first instar larvae (> 100) were placed into the center of the fruit. First instars were chosen as this stage, along with eggs, was observed in this experiment to have the greatest amount of tolerance to the fumigant (see Results). The exact number per treatment was not determined. The flavedo/albedo plug was then replaced and taped over. As time would be needed for the fumigant to penetrate the fruit, the fumigations were conducted for 8 and 12 h. Doses were given so to obtain CXT doses that would be comparable to CXTs obtained in the lemon quality portion of this research. After fumigation, the COS was flushed from the jars with 5 min of airflow and the open jars allowed to stand in a hood for 2 h for aeration. The lemons were then sliced, the larvae removed from the fruit by rinsing with water and placed on a larval diet for 7–10 days at 26°C. To ensure that no living larvae remained in the lemons the sliced-open fruit was held in a screened drinking cup over sand. After counting the number of pupae following incubation, the estimated population was calculated based upon the number of pupae in the controls and then used to calculate percent mortality in the treatments.

Data analysis

The experiment was set up as a completely randomized design. Analysis of variance was calculated using SuperANOVA (Abacus Concepts, Berkeley, CA) and mean separations by Duncan's new multiple range test.

Results

Following fumigation, the lemons were very malodorous and remained somewhat so even after 24 h of aeration and only after 48 h did the off-odor decline below sensory levels. Rind injury, consisting mostly of pitting and browning, was very slight and not different from the controls at fumigation durations of 4 and 8 h, but became increasingly apparent at durations of 12 h and longer (*Figure 2*). Especially prevalent was a browning of the stylar end of the fruit. The amount of injury sustained by the 16 and 20 h fumigation treatments was of a severity sufficient to render the fruit unfit for marketing.

With the exception of the 16-h treatment, firmness was not significantly affected by fumigation (*Table 1*). Similarly, although there was a trend toward increased weight loss by the fumigated fruit, none of the differences were statistically different from the controls.

Fumigation had little effect on the pH, acidity or soluble solid content of lemon juice (*Table 1*). One very noticeable effect of fumigation on the juice, however, was the presence of an objectionable odor emanating from the juice of lemons fumigated for 12 h or longer. After 12 h of fumigation, however, the odor was barely noticeable and would probably have

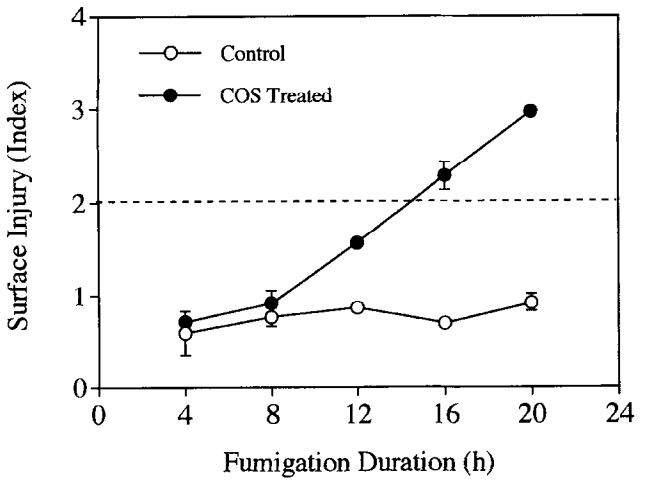

Figure 2. Surface injury index in response to different durations of COS fumigation. Initial starting dose was 70 mg l^{-1}. The dotted line indicates the injury level above which the fruit were considered to be unmarketable. Each data point represents three replicates. Bars are standard errors and if not visible are hidden by a data point

no influence on marketability, but it was strong following 20 h of COS fumigation.

Fruit color was strongly influenced by fumigation. As shown in *Table 1*, the values for lightness were much lower, indicating that a darkening had taken place due to fumigation. Only the 16-h treatment was not statistically different from the control, this probably being due to a variability in the fruit of that treatment. The darkening was even more pronounced immediately following fumigation, when the lemons appeared to have a somewhat greenish cast (data not shown). Hue angle, the other color measurement taken, was not as strongly influenced as lightness, and was only significantly different in the 4- and 8-h treatment (*Table 1*). In these cases the hue angle increased, the coloration moving from yellow slightly towards green.

Table 1. Lemon quality characteristics following COS fumigation

		Hours of fumigation (CXT)[b]				
Character[a]	COS	4 (242)	8(428)	12(565)	16(667)	20(751)
Firmness[c] (mm)	–	2.93	3.05	3.16	3.13	3.15
	+	3.19	3.15	3.26	3.26*	3.14
Weight loss (%)	–	6.46	6.58		6.54	6.95
	+	6.94	6.85		7.29	7.83
pH	–	2.58	2.65	2.68	2.62	2.52
	+	2.59	2.68	2.72	2.67	2.57*
Acids (%)	–	5.95	5.74	5.78	5.87	5.97
	+	6.03	5.67	5.73	5.52	5.55
Soluble solids (%)	–	6.80	7.13	6.97	7.03	7.20
	+	7.10*	6.77	7.00	7.00	6.83
Color[d] (lightness)	–	77.25	76.21		76.32	77.05
	+	75.75**	74.76*		74.93	75.08**
(hue angle)	–	91.53	91.13		90.43	92.26
	+	91.17*	90.66*		90.58	91.91

[a]Statistical comparisons were made for each character between the control (no COS) and COS treatments within each fumigation treatment. Values that are significantly different at $P \leq 0.05$ and $P \leq 0.01$ are indicated by one or two stars, respectively.
[b]Initial target dose = 70 mg l^{-1}. CXT = dose concentration × time.
[c]Firmness is measured in cm displacement of the compression anvil. More displacement indicates softer fruit.
[d]Values from colorimeter. A lower value for lightness indicates darker fruit coloration. Hue angle is in degrees (0° = red, 90° = yellow, 180° = green).

Mortalities of exposed MFF eggs and larvae exposed to COS are reported in *Tables 2 3* and *4*. With both eggs and larvae it was found that treatment duration was more important than CXT value. For instance, eggs in experiment 2 (*Table 2*) were only affected to a small degree (15% mortality) with a dose of 100 mg l^{-1} for 4 h but were all killed at a dose of 60 mg l^{-1} for 6 h, even though the CXT value was less at 6 h. Third instar larvae were somewhat more susceptible to COS than eggs and first instar larvae, 4 h being sufficient to achieve 100% mortality. First instars and eggs had a similar tolerance to COS.

Fruit fly larvae placed inside lemons and exposed to COS were all killed at doses of 42 or 55 mg l^{-1} for 12 h (*Table 5*). The lowest dose with a 12 h exposure (29 mg l^{-1}) and all three of the doses for 8 h did not achieve complete kill.

Discussion

Lemons tolerated COS at a dose of 70 mg l^{-1} for 8 h (CXT = 428 mg h l^{-1}) with no noticeable loss in quality and for 12 h (CXT = 565 mg h l^{-1}) with only very slight injury to the peel. Increasing the fumigation duration beyond this point, however, lead to a persisting off-odor in the juice and an increased incidence of peel injury. A darkening in the coloration of the peel was detectable at all doses, but was not readily visible to the eye following 4 weeks of storage.

Our tests on exposure durations that would be needed to kill MFF indicated that long fumigations would be likely to be necessary to ensure a complete kill. This is in agreement with the work of Banks *et al.* (1993), who found that even at relatively high CXT doses of up to 600 mg h l^{-1}, a 6 h fumigation was not completely effective in controling Queensland fruit fly.

A COS dose of 42 mg l^{-1} for 12 h (CXT = 430 mg h l^{-1}) was the lowest dose tested in which we achieved 100% mortality with first instar larvae placed into lemons. Since our fumigation experiments with exposed insects had shown that third instars were the least tolerant to COS and that eggs and first instars had similar tolerances to COS, this dose would be able to kill any lifestage of MFF located within the lemon. This lethal dose can be compared with data from the lemon quality portion of this research in which a 12-h fumigation with a higher CXT of 565 mg h l^{-1} had no significant effect on lemon quality besides very slight rind injury. This indicates that COS could be used as a fumigant to disinfest lemons of MFF and lemon quality would still be maintained. In addition, further experimentation could be carried out to more precisely determine and perhaps further reduce the needed dose.

It should be noted that although lemons are believed to be a very low risk in terms of causing an infestation of MFF (Spitler *et al.*, 1984), they are nonetheless still subject to quarantine restrictions for this insect, one of the most important pests in California. If methyl bromide was withdrawn from use then COS might be a useful quarantine option.

Table 2. Mortality of exposed Mediteranean fruit fly eggs following different doses and durations of carbonyl sulfide fumigation

		Experiment 1		Experiment 2	
Duration (h)	Dose[a] (mg l^{-1})	CXT[b] (mg h l^{-1})	Mortality[c] (%)	CXT (mg h l^{-1})	Mortality (%)
2	100	198	2.9 (2.9)		
2	200	397	25.1 (9.7)	402	9.7 (1.2)
4	40			157	4.3 (2.7)
4	60			246	17.5 (2.5)
4	80			323	17.5 (8.9)
4	100	385	99.2 (0.4)	401	15.0 (4.5)
4	200	796	100.0 (0.0)	769	53.1 (5.5)
6	40			220	97.0 (3.0)
6	60			362	100.0 (0.0)
6	80			468	100.0 (0.0)
6	100	568	100.0 (0.0)	588	100.0 (0.0)
6	200	1177	100.0 (0.0)		

[a]Initial target dose.
[b]CXT = dose concentration × time.
[c]Abbott's corrected % mortality (Abbott, 1925). Correction based upon a mortality of 18% for experiment 1 and 19% for experiment 2 untreated controls. Values are means of three replications with 100 eggs per replication. Numbers in parentheses are standard errors.

Table 3. Mortality of exposed first instar Mediteranean fruit fly larvae following different doses and durations of carbonyl sulfide fumigation

		Experiment 1		Experiment 2	
Duration (h)	Dose[a] (mg l^{-1})	CXT[b] (mg h l^{-1})	Mortality[c] (%)	CXT (mg h l^{-1})	Mortality (%)
4	100	388	94.9 (2.6)		
4	200	804	94.9 (2.6)	795	91.0 (1.8)
4	300			1180	86.5 (3.2)
6	20			122	50.0 (7.2)
6	40			228	99.3 (0.7)
6	60			355	100.0 (0.0)
6	80			470	99.3 (0.7)
6	100			585	100.0 (0.0)

[a]Initial target dose.
[b]CXT = dose concentration × time.
[c]Numbers based on estimated treated population calculated from control means. For experiments 1 and 2, the mean number of living pupae in the controls was 13 and 48, respectively. All values are based on three replications. Numbers in parentheses are standard errors.

Table 4. Mortality of exposed third instar Mediteranean fruit fly larvae following different doses and durations of carbonyl sulfide fumigation

		Experiment 1		Experiment 2	
Duration (h)	Dose[a] (mg l^{-1})	CXT[b] (mg h l^{-1})	Mortality[c] (%)	CXT (mg h l^{-1})	Mortality (%)
2	100	198	58.14 (5.6)		
2	200	397	58.14 (8.4)	403	53.0 (11.6)
2	300			601	64.0 (8.9)
4	20			85	3.5 (2.6)
4	40			162	89.4 (4.3)
4	60			246	100.0 (0.0)
4	80			320	90.1 (9.9)
4	100			403	95.7 (4.3)
4	120			461	100.0 (0.0)
4	140			547	100.0 (0.0)
4	200	797	100.0 (0.0)		
6	100	568	100.0 (0.0)		
6	200	1177	100.0 (0.0)		

[a]Initial target dose.
[b]CXT = dose concentration × time.
[c]Abbott's corrected % mortality (Abbott, 1925). Correction based upon a mortality of 14% for experiment 1 and 6% for experiment 2 untreated controls. Values are means of three replications with 50 larvae per replication. Numbers in parentheses are standard errors.

Table 5. Mortality of Mediterranean fruit fly first instar larvae in lemons exposed to different doses of carbonyl sulfide

Duration (h)	Dose[a] (mg l^{-1})	CXT[b] (mg h l^{-1})	Mortality[c] (%)
8	42	300	99.6 (0.4)
8	61	444	71.4 (20.3)
8	81	585	95.3 (3.2)
12	29	278	96.4 (3.6)
12	42	430	100.0 (0.0)
12	55	560	100.0 (0.0)

[a]Initial target dose.
[b]CXT = dose concentration × time.
[c]Numbers based on estimated treated population calculated from control mean. The mean number of living pupae in the control was 28. All values are based on three replications. Numbers in parentheses are standard errors.

There are, however, two drawbacks that would hinder its acceptance as a viable quarantine treatment. The first is the lengthy time that is required for COS to kill MFF. Our fumigation tests with exposed insects indicated that at least 6 h would probably be required and our in-fruit tests point to even longer times being needed, while a standard methyl bromide dose for fruit fly requires only 2 h (Anon, 1976). The second is the strong off-odor that persists for days following fumigation, even with aeration. These two factors would result in added costs to the use of the fumigant and may make it not cost effective. In light of this, COS may have a greater potential for use against other, more susceptible insects or in combination treatments that would allow for shorter fumigation duration.

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